

AFFIDAVIT

Ivan A. Casas, being first duly sworn, deposes and says as follows:

1. I am an employee of Biogaia Biologics, Inc., which is affiliated with Biogaia Biologics AB.
2. I am familiar with the application, Serial No. 476,630 of inventors Walter J. Dobrogosz and Sven E. Lindgren (the Application) owned by Biogaia Biologics AB, and with the Office Action of December 19, 1996 in the Application.
3. As shown on the attached copy of my resume, I have been working professionally in the field of microbiology for nearly thirty years. Since 1985, I have worked with *Lactobacillus* species. My work in microbiology includes studies of gastrointestinal colonization in various animals, including chickens, turkeys, mice, rats, pigs and humans.
4. As part of my studies on colonization, I have utilized the now-patented technique disclosed in the Application for determining the presence of *Lactobacillus reuteri* (U.S. Patent No. 5,352,586, issued October 4, 1994) for studying colonization of the gastrointestinal tracts of animals by strains of this species. This technique provides an unequivocal demonstration that colonization has occurred. In any animal system, one can use this method to determine the number of viable cells of *Lactobacillus reuteri* per gram of feces as an indicator of whether colonization has occurred after a particular treatment of the animal. Unlike the determination of many other microorganisms which might be fed to animals or which might occur in the feces generally, this technique is rapid, accurate and sensitive.
4. There have been numerous studies in which animal colonization has been determined, both with *L. reuteri* and with other species. Examples of reports in the literature of some of these studies, both by people in my laboratory and by others, are listed below, copies of which are attached:

Casas, IA, FW Edens, WJ Dobrogosz and CR Parkhurst. 1993. Performance of GAIAspray and GAIAspray: A *Lactobacillus reuteri*-based Probiotic for Poultry. In: Prevention and Control of Potentially Pathogenic Microorganisms in Poultry and Poultry Meat Products, Proc. 12, FLAIR No. 6, Probiotics and Pathogenicity, pp 63-71. Eds. Jensen, JF, MH Hinton, and RWA Mulder. DLO Centre for Poultry Research and Information Services, Beekbergen, The Netherlands.

Casas, IA, FW Edens and WJ Dobrogosz. 1997. *Lactobacillus reuteri*: An

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Efective Probiotic for Poultry, Other Animals and Humans. In Lactic Acid Bacteria, 2nd Ed., edited by S. Salminen and A von Wright

Edens, FW, CR Parkhurst, IA Casas and WJ Dobrogosz. 1997. Principles of *Ex Ovo* Competitive Exclusion and *In Ovo* Administration of *Lactobacillus reuteri*. Poultry Sci. 76:179-196.

England, JA, SE Watkins, E Saleh, and PW Waldroup. 1996. Effects of *Lactobacillus reuteri* on Live Performance and Intestinal Development of Male Turkeys. Appl. Poultry Sci. 5:311-324.

Saxlin M. 1996. Colonization of the Human Gastrointestinal Tract by Probiotic Bacteria. Nutri. Today Suppl. 31:SS-8S.

Shornikova, A-V, IA Casas, E Isolauri, H Mykkanen and T. Vesikari. 1997. *Lactobacillus reuteri* as a Therapeutic Agent in Acute Diarrhea in Young Children. J. Pediatric AGastroenterology & Nutr. (n press).

Wolf, BW, KA Garleb, DG Ataya and IA Casas. 1995. Safety and Tolerance of *Lactobacillus reuteri* in Healthy Adult Male Subjects, Microbial Ecol. in Health & Dis. 8:41-50.

5. It is my professional opinion that anyone who is familiar with the disclosure in the Application and with working with a particular animal or animals, could easily determine how many cells of *L. reuteri* to be used in the particular animal(s) to result in colonization, by doing a preliminary survey with varying dose levels, and analyzing the feces using the method reported in the Application. Of course, in the alternative, a large dose could be used, for example, 10⁹/gram or even more, to assure colonization without preliminary testing in any system.
6. The above statements are true to the best of my knowledge, information and belief.

This the 12 day of June, 1997.


Ivan A. Casas-Perez

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STATE OF NORTH CAROLINA
COUNTY OF Wake

Before me personally appeared Ivan A. Casas-Perez, known to me, who personally subscribed his name to the foregoing document in my presence and made oath before me that the statements set forth therein were true to the best of his knowledge, information and belief, this 12th day of June, 1997.

This the 12th day of June, 1997.

June Potter
Notary Public

My Commission Expires: 10/22/99

I. A. Casas Resumé

IVAN A. CASAS

Director of Research, Biogaia Biologics AB, Sweden.

HIGHER EDUCATION:

Ph.D. in Microbiology. The Pennsylvania State University, University Park, PA. 1968.
Biochemistry and Animal Nutrition. Universidad Agraria La Molina, Lima. Peru; M. S. level. 1963

Ag. Engineer. Universidad Agraria La Molina, Lima. Peru, 1962

B.S. in Ag. Sciences. Universidad Agraria La Molina, Lima. Peru, 1961

PROFESSIONAL EXPERIENCE:

Director of Research. Biogaia Biologics, AB, Sweden	Sept 1, 1989 -present
Director of Research. ProBiologics International, Inc., Raleigh, NC.	Sept 1, 1987-1989
Adjunct Professor of Microbiology. North Carolina State University, Raleigh, NC.	Sept 1, 1987-present
Visiting Professor of Microbiology. 1987 North Carolina State University, Raleigh, NC.	July 31, 1985-Sept 1, 1988
Professor. School of Veterinary Sciences, Universidad del Zulia, Maracaibo, Venezuela.	1979-1988
Visiting Professor of Microbiology. The Pennsylvania State University, University Park, PA.	1977-1978
Associate Professor. School of Veterinary Sciences, Universidad del Zulia, Maracaibo, Venezuela.	1970-1979
Assistant Professor. Universidad de Oriente. Maturin and School of Veterinary Sciences, Universidad del Zulia, Maracaibo, Venezuela.	1968-1970

PROFESSIONAL HONORS AND AWARDS:

FAO fellowship (1965-1967), Penn State Univ; Assist. Biochemist, Nuclear Energy Program, Org. Am. States (Costa Rica).

Postdoctoral fellow (USDA research grant), Dept. Microbiol., NC State Univ. (Raleigh, NC).

Member of: American Society for Microbiology
Institute of Food Technologists
Poultry Science Association
Sociedad Venezolana de Microbiología.

SELECTED PERSONAL PUBLICATIONS AND PRESENTATIONS (OF ca 20). In 1985 Dr. Casas changed his field of study from plant -host interactions and food microbiology (exemplified by publications 1 and 2) to research on Lactobacillus species as indicated:

Currently in various stages of progress are 9 manuscripts summarizing the studies showing:

- 1- The beneficial effects of Lactobacillus reuteri administrations on livibility, morbidity, and commercial productivity in chickens and turkeys.
- 2- Studies on the incorporation of Lactobacillus reuteri in foods, including survival, effect on product characteristics.
- 3- The beneficial effects of Lactobacillus reuteri administrations on humans.

PUBLICATIONS (LAST 8 YEARS):

Casas I. A., Edens, F.W. and Dobrogosz, W.J. *Lactobacillus reuteri: An Effective Probiotic for Poultry, Other Animals and Humans.* In: *Lactic Acid bacteria*, (S. Salminen and A. von Wright, Eds.). (In Press) (1997).

Shornikova A., Casas I. A., Isolauri E., Mykkanen H. and Vesikari T. *Lactobacillus reuteri as a Therapeutic Agent in Acute Diarrhoea in Young Children.* *J. Pediatric Gastroenterol. and Nutr.* (In Press) (1997).

Edens, F. W., Parkhurst, C. R., Casas I. A. and Dobrogosz, W. J. *Principles of Ex Ovo Competitive Exclusion and In Ovo Administration of Lactobacillus reuteri.* *Poultry Science* 76:179-196. (1997).

England, J. A., Watkins, S. E., Saleh, E., Waldroup P. W., Casas I. and Burnham, D. *Effects of Lactobacillus reuteri Hatchery Spray and Feed Application on Live Performance and Intestinal Development of Male Turkey.* *J. Applied Poultry Res.* 5:311-324. (1996)

Casas I. A., Edens, F. W., Kreuger, K. James, R., Parkhurst, C. R. and Dobrogosz, W. J. *Protection Against Avian Growth Depression in Commercial Turkey Production by Lactobacillus reuteri Prophylaxis.* (Manuscript submitted for publication).

Wolf B. W., Garleb K. A., Ataya D. G. and Casas I. A. *Safety and Tolerance of Lactobacillus reuteri in Healthy Adult Male Subjects.* *Microbial Ecology in Health and Disease*, 8: 41-50 (1995).

Casas, I. A., Edens F. W., Dobrogosz W. J., and Parkhurst C. R. *Performance of GAIAspray™ and GAIAspray™: a Lactobacillus reuteri-based probiotics for poultry. models for probiotic evaluation.* In: *Prevention and Control of Potentially Pathogenic Microorganisms in Poultry and Poultry Meat Products, Proceedings 12, FLAIR No. 6. Probiotics and Pathogenicity*, pp 63-71. Eds: Jensen J. F., Hinton M. H. and Mulder R. W. A. W. DLO Centre for Poultry Research and Information Services, Beekbergen, The Netherlands (1993)

Gomez, G., Casas, I. and Dobrogosz, W. *Evaluation of the Effect of Lactobacillus reuteri on Non-Infected and Cryptosporidium parvum-Infected, Artificially-Reared Piglets.* North Carolina Experiment Station Research Report, p 10 (1993).

Speck ML, Dobrogosz WJ, Casas I. A. *Lactobacillus reuteri in food suplementation.* *Food Technology*, July: pp. 90-94. (1993).

Arhne, S., Casas. I.A., S. E. Lindgren, G. Molin, and W.J. Dobrogosz. *Spontaneous and SDS-induced phenotype and plasmid alterations in starter cultures of Lactobacillus plantarum.* *System. Appl. Microbiol.* 15: 285-288. (1992).

Dobrogosz W. J., Casas I. A., Pagano G. A., Talarico T. L., Sjorberg B-M and Karlson M. *Lactobacillus reuteri and the Enteric Microbiota.* In: *The regulatory and Protective Role of the Normal Microflora* (Eds.: Grubb R., Midtevedt T. and Norin E.) Macmillan LTD, London, pp. 283-292 (1989).

Talarico T. L., Casas I. A., Chung T. C. and Dobrogosz W. J. *Production and Isolation of Reuterin, A Growth Inhibitor Produced by Lactobacillus reuteri.* *Antimicrob. Agents Chemotherapy* 32:1854-1858 (1988).

Casas, I. A., E. Marquez and L. Zimmerman. *Expression of Hemolysin Trait in a proteolytic Transconjugant of Streptococcus faecalis.* *Biochemie.*70: 283-285. (1988).

Cosby, W., I. Casas and W. J. Dobrogosz. *Formation, Regeneration and Transfection of Lactobacillus plantarum Protoplasts.* *Applied and Environmental Microbiology.* 54 (11): 2599-2602. (1988).

PRESENTATIONS (LAST 4 YEARS):

Casas I. A., Shornikova A., Isolauri E., Salminen S. and Vesikari T. *Colonization of Lactobacillus reuteri in the Gastrointestinal Tract of Children.* *World Congress on Anaerobic Bacteria and Infections.* San Juan Puerto Rico, November 5-8, 1995. Abstracts, p 36 (1995).

Edens F. W., I. A. Casas, and C. R. Parkhurst and Kristi Joyce. Reduction of Egg-Borne *E. coli*-Associated Chick Mortality by In-Hatcher exposure to *Lactobacillus reuteri*. *Poultry Science* 73: (Sup. 1, Abs.), p 79. (1994).

Edens F. W., I. A. Casas, and C. R. Parkhurst. Effect of *Lactobacillus reuteri* as GAIA Spray™ on In-Pen and In-Hatcher Lateral Transmission of *Salmonella typhimurium* and Enteropathogenic *Escherichia coli* in chickens. Lactic '94, Caen, France. September 7-10, (1994)

Casas I.A., Edens F.W., Dobrogosz W.J. and Parkhurst C.R. Control of *Salmonella typhimurium* GI tract Infection by GAIAfeed™ and GAIAspray™ in Presence of *Staphylococcus*. *Poultry Science* 73: (Sup. 1, Abs.), p 131. (1994).

Casas I.A., Edens F.W., Dobrogosz W.J. and Parkhurst C.R. Avian Models for Probiotic Evaluation. Lactic '94, Caen, France. September 7-10, (1994)

Wolf B.W., Garleb K.A., Casas I.A. Safety and tolerance of *Lactobacillus reuteri* in healthy adult male subjects (Abst.). XIX International Congress on Microbial Ecology and Disease, Sept. 18-21, ISTISAN Congressi 37, Rome, Italy. (1994).

Casas I.A., Edens F.W. and Parkhurst C.R. GAIAfeed™ in commercial turkey production: Improved field performance. *Poultry Science* 72: (Sup. 1, Abs.), p 158. (1993)

Edens F.W., Casas I.A., and Parkhurst C.R. GAIAfeed™ in commercial turkey production: II Influence of *Salmonella* on viability. *Poultry Science* 72: (Sup. 1, Abs.), p 163. (1993)

Edens F. W., I. A. Casas, and C. R. Parkhurst. Effect of *Lactobacillus reuteri* as GAIA Spray™ on In-Pen and In-Hatcher Lateral Transmission of *Salmonella typhimurium* and Enteropathogenic *Escherichia coli* in chickens. *Poultry Science* 72: (Sup. 1, Abs.), p 63. (1993).

Dobrogosz W. J., Dunham H. J., Edens F. W. and Casas I. A. Immunomodulation of stressor-associated in neonate chickens and turkeys by oral administration of *Lactobacillus reuteri*. *Poultry Science* 72: (Sup. 1, Abs.), p 163. (1993).

Dunham H. J., Williams H., Edens F. W., Casas I. A. and Dobrogosz W.J. *Lactobacillus reuteri* immunomodulation of stressor-associated diseases in newly hatched chickens and turkeys. *Poultry Science* 72: (Sup. 1, Abs.), p 103. (1993).

Edens, F. W., Phelps, P. V., Parkhurst, C. R., and Casas, I. A. Early Cecal Colonization by *Lactobacillus reuteri* in Chicks and Poulets Promoted by *In Ovo* Inoculation and Post-Hatch Spray. Southern Poultry Science Society Annual Meeting. pp 14. (1992)

Edens, F. W., Parkhurst, C. R., and Casas, I. A. GAIAfeed™ Supplementation to Market Turkeys Improves Performance. Poultry Science Association Annual Meeting. Fayetteville AK. August 3-6, (1992).

Dobrogosz, W. J., Dunham H. J., Edens, F. W., and Casas, I. A. Immunomodulation of Stressor-associated Diseases in Neonate Chicken and Turkeys. International Symposium of Intestinal Microecology (Helsinki, Finland) and the 8th International Congress of Immunology (Budapest, Hungary). (1992).

Edens, F. W., Parkhurst, C. R., and Casas, I. A. GAIAfeed™ Improves Turkey Performance. XIX World's Poultry Congress. Amsterdam, September 20-22. (1992).

Dobrogosz, W. J., Black, B. L., and Casas, I. A. 1991. Delivery of Viable *Lactobacillus reuteri* to the Gastrointestinal Tract of Poultry. *Poultry Science*. 70 Supplement 1: pp 158.

Edens, F. W., Parkhurst, C. R., and Casas, I. A. *Lactobacillus reuteri* and Whey Reduce *Salmonella* Colonization in the Ceca of Turkey Poulets. *Poultry Science*. 70 Supplement 1: pp 158. (1991).

Parkhurst, C. R., Edens, F. W., and Casas, I. A. *Lactobacillus reuteri* and Dietary Whey Effect on Twenty Day Body Weights of Turkey Poulets Subjected to either Cold or Low Protein Stress. *Poultry Science*. 70 Supplement 1: pp 173. (1991).

Edens, F. W., Parkhurst, C. R., and Casas, I. A. *Lactobacillus reuteri* Feeding Elevates Levels of Blood Constituents, Hematocrit and Hemoglobin in Chicks and Poulets. *Poultry Science*. 70 Supplement 1: pp 37. (1991).

Edens, F. W., Casas, I. A., and Dobrogosz, W. J. In Ovo, Spray, and Feed Supplement Methods for Delivering Lactobacillus reuteri (a Direct Fed Microbial) to Turkey Embryos and Newly Hatched Poulets. Lactic '91, a European Symposium on Lactic Acid Bacteria: Research and Industrial Applications in the Agro-Food Industries. Caen, Normandy, France, September 12-13. Abstracts. pp 2-6. (1991).

Edens, F. W., Parkhurst, C. R., and Casas, I. A. Reduced Salmonella Colonization in the Ceca of Turkey Poulets by Lactobacillus reuteri and Whey. Lactic '91, a European Symposium on Lactic Acid Bacteria: Research and Industrial Applications in the Agro-Food Industries. Caen, Normandy, France, September 12-13. Abstracts. pp 2. (1991).

Parkhurst, C. R., Edens, F. W., and Casas, I. A. Lactobacillus reuteri Amelioration of Cold but not Low Dietary Protein Stress-Induced Growth Depressions in Turkey Poulets. Lactic '91, a European Symposium on Lactic Acid Bacteria: Research and Industrial Applications in the Agro-Food Industries. Caen, Normandy, France, September 12-13. Abstracts. pp 2-8. (1991).

Casas, I. A., Edens, F. W., and Parkhurst, C. R. GAIAfeed™ and GAIA Spray™ Effect on Body Weights and Mortality of Serviced Turkey Poulets Subjected to Mild Cold Stress and "Natural" Bacterial Pathogen Stress. Lactic '91, a European Symposium on Lactic Acid Bacteria: Research and Industrial Applications in the Agro-Food Industries. Caen, Normandy, France, September 12-13. Abstracts. pp 2-9. (1991).

Cosby, W. M., I. Casas, and W. J. Dobrogosz. Formation, Regeneration, and Transfection of Lactobacillus plantarum Protoplasts. ASM Annual Meeting Abstracts. pp 154. (1988).

Casas, I. A., Jeffrey, S., Shrago, A. W., and Dobrogosz, W. J. Spontaneous Plasmid Rearrangements and Genesis in Lactobacillus plantarum. FEMS Microbiology Reviews 46 (3): p.18. (1987).

Casas, I. A. and Dobrogosz, W. J. Variants in Lactobacillus plantarum: Effect of Cultural and Nutritional Conditions. ASM Annual Meeting Abstracts. pp 173. (1987).